Amendments to the Claims

Applicants have amended claims 1, 7, 18, 19, 23 and 32 without any intention of disclaiming equivalents thereof. The Examiner has withdrawn claims 11, 15, 26-30, and 128. The following list of claims replaces all prior versions and lists of claims in the application.

What is claimed is:

1. (Currently amended) A method for detecting the presence of a post-translational modification on a target protein within a sample, comprising:

(1) computationally analyzing amino acid sequence of said target protein to identify one or more candidate ~~site~~ sites for said post-translational modification;

(2) computationally identifying the amino acid sequence of one or more fragments of said target protein, said fragment predictably ~~results~~ resulting from a treatment of said target protein within said sample, and said fragment ~~encompasses~~ comprising said potential post-translational modification site and a PET (proteome epitope tag) unique to said fragment within said sample;

(3) generating a capture agent that specifically binds said PET, and immobilizing said capture agent to a support;

(4) subjecting said sample to ~~said~~ a treatment to render said fragment soluble in solution, and contacting said sample after said treatment ~~to~~ with said capture agent;

(5) detecting, on said fragment bound to said capture agent, the presence or absence of said post-translational modification by using a secondary capture agent specific for said post-translational modification labeled with a detectable moiety.

2. (Original) The method of claim 1, wherein said post-translational modification is acetylation, amidation, deamidation, prenylation, formylation, glycosylation, hydroxylation, methylation, myristoylation, phosphorylation, ubiquitination, ribosylation or sulphation.

3. (Original) The method of claim 2, wherein said post-translational modification is

phosphorylation on tyrosine, serine or threonine.

4. (Original) The method of claim 1, wherein said step of computationally analyzing amino acid sequences includes a Nearest-Neighbor Analysis that identifies said PET based on criteria that also include one or more of pI, charge, steric, solubility, hydrophobicity, polarity and solvent exposed area.

5. (Original) The method of claim 4, further comprising determining the specificity of said capture agent generated in (3) against one or more nearest neighbor(s), if any, of said PET.

6. (Original) The method of claim 5, wherein peptide competition assay is used in determining the specificity of said capture agent generated in (3) against said nearest neighbor(s) of said PET.

7. (Currently amended) The method of claim 1, wherein said step of computationally analyzing amino acid sequences includes a solubility analysis that identifies a said PET that ~~are~~ is predicted to have at least a threshold solubility under a designated solution condition.

8. (Original) The method of claim 1, wherein the length of said PET is selected from 5-10 amino acids, 10-15 amino acids, 15-20 amino acids, 20-25 amino acids, 25-30 amino acids, or 30-40 amino acids.

9. (Original) The method of claim 1, wherein said capture agent is a full-length antibody, or a functional antibody fragment selected from: an Fab fragment, an $F(ab')_2$ fragment, an Fd fragment, an Fv fragment, a dAb fragment, an isolated complementarity determining region (CDR), a single chain antibody (scFv), or derivative thereof.

10. (Original) The method of claim 1, wherein said capture agent is nucleotides; nucleic acids; PNA (peptide nucleic acids); proteins; peptides; carbohydrates; artificial polymers; or small organic molecules.

11. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 1, wherein said capture agent is aptamers, scaffolded peptides, or small organic molecules.

12. (Original) The method of claim 1, wherein said treatment is denaturation and/or fragmentation of said sample by a protease, a chemical agent, physical shearing, or sonication.

13. (Original) The method of claim 12, wherein said denaturation is thermo-denaturation or chemical denaturation.

14. (Original) The method of claim 13, wherein said thermo-denaturation is followed by or concurrent with proteolysis using thermo-stable proteases.

15. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 13, wherein said thermo-denaturation comprises two or more cycles of thermo-denaturation followed by protease digestion.

16. (Original) The method of claim 12, wherein said fragmentation is carried out by a protease selected from trypsin, chymotrypsin, pepsin, papain, carboxypeptidase, calpain, subtilisin, gluc-C, endo lys-C, or proteinase K.

17. (Original) The method of claim 1, wherein said sample is a body fluid selected from: saliva, mucous, sweat, whole blood, serum, urine, amniotic fluid, genital fluid, fecal material, marrow, plasma, spinal fluid, pericardial fluid, gastric fluid, abdominal fluid, peritoneal fluid, pleural fluid, synovial fluid, cyst fluid, cerebrospinal fluid, lung lavage fluid, lymphatic fluid, tears, prostatitc fluid, extraction from other body parts, or secretion from other glands; or from supernatant, whole cell lysate, or cell fraction obtained by lysis and fractionation of cellular material, extract or fraction of cells obtained directly from a biological entity or cells grown in an artificial environment.

18. (Currently amended) The method of claim 1, wherein said sample is obtained from human, mouse, rat, frog ~~(Xenopus)~~, fish ~~(zebra fish)~~, fly ~~(*Drosophila melanogaster*)~~, nematode ~~(*C. elegans*)~~, fission or budding yeast, or plant ~~(*Arabidopsis thaliana*)~~.

19. (Currently amended) The method of claim 1, wherein said sample ~~is produced by treatment of~~ comprises membrane bound proteins.

20. (Original) The method of claim 1, wherein said treatment is carried out under conditions to preserve said post-translational modification.

21. (Original) The method of claim 1, wherein said PET and said candidate site for said post-translational modification do not overlap.

22. (Original) The method of claim 1, wherein said capture agent is optimized for selectivity for said PET under denaturing conditions.

23. (Currently amended) The method of claim 1, wherein ~~step (5) is effectuated by using a secondary capture agent specific for said post-translational modification, wherein~~ said secondary capture agent is labeled by a detectable moiety selected from: an enzyme, a fluorescent label, a stainable dye, a chemilumninescent compound, a colloidal particle, a radioactive isotope, a near-infrared dye, a DNA dendrimer, a water-soluble quantum dot, a latex bead, a selenium particle, or a europium nanoparticle.

24. (Original) The method of claim 23, wherein said post-translational modification is phosphorylation, and said secondary capture agent is a labeled secondary antibody specific for phosphorylated tyrosine, phosphorylated serine, or phosphorylated threonine.

25. (Original) The method of claim 24, wherein said secondary antibody is labeled by an enzyme

or a fluorescent group.

26. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 25, wherein said enzyme is HRP (horse radish peroxidase).

27. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 23, wherein said post-translational modification is phosphorylation, and said secondary capture agent is a fluorescent dye that specifically stains phosphoamino acids.

28. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 27, wherein said fluorescent dye is Pro-Q Diamond dye.

29. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 23, wherein said post-translational modification is glycosylation, and said labeled secondary capture agent is a labeled lectin specific for one or more sugar moieties attached to the glycosylation site.

30. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 23, wherein said post-translational modification is ubiquitination, and said labeled secondary capture agent is a labeled secondary antibody specific for ubiquitin.

31. (Original) The method of claim 1, wherein said sample contains billion molar excess of unrelated proteins or fragments thereof relative to said fragment.

32. (Currently amended) The method of claim 1, further comprising ~~qauntitating~~ quantitating the amount of said fragment bound to said capture agent.

33. (Original) The method of claim 1, wherein step (3) is effectuated by immunizing an animal with an antigen comprising said PET sequence.

34. (Original) The method of claim 33, wherein the N- or C-terminus, or both, of said PET sequence are blocked to eliminate free N- or C-terminus, or both.

35. (Original) The method of claim 34, wherein the N- or C-terminus of said PET sequence are blocked by fusing the PET sequence to a heterologous carrier polypeptide, or blocked by a small chemical group.

36.- 125. (Cancelled by applicants in response to restriction requirement).

126. (Previously Presented) The method of claim 3, wherein said post-translational modification is phosphorylation on tyrosine.

127. (Previously presented) The method of claim 24, wherein said post-translational modification is phosphorylation, and said secondary capture agent is a labeled secondary antibody specific for phosphorylated tyrosine.

128. (Withdrawn by examiner pursuant to requirement for election of species) The method of claim 23, wherein said post-translational modification is phosphorylation, and said secondary capture agent is a fluorescent dye that specifically stains phosphorylated tyrosine.

129. (Previously presented) The method of claim 9, wherein said capture agent is a full-length antibody.

130. (Previously presented) The method of claim 23, wherein said secondary capture agent is labeled by a fluorescent label.

131. (Previously presented) The method of claim 25, wherein said secondary antibody is labeled by a fluorescent group.

132. (Previously presented) The method of claim 127, wherein said secondary antibody is labeled by a fluorescent group.